

Chromosome Numbers, Spores and Sporangial Annuli of *Gymnocarpium oyamense* (Woodsiaceae) and the Inferred Cytogeography

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Cytological observations of the Japanese *Gymnocarpium oyamense* revealed the presence of diploids, triploids and tetraploids ($x = 40$). Diploids have smaller spores than tetraploids. The Japanese diploids and plants suspected of being diploids because of their small spores ($<34\ \mu\text{m}$) occur in the Kanto district and disjunctly in Mie Prefecture. Tetraploids and presumed tetraploids with large spores ($>35\ \mu\text{m}$) are widely distributed from western Kanto westward to Shikoku, and are allopatric with the diploids. In China, suspected diploids are widely distributed in the inland provinces of Henan, Sichuan, Yunnan, Shaanxi and Gansu; tetraploids are mainly in the eastern provinces of Anhui, Jiangxi, Hunan, Guizhou, and Sichuan. The Japanese plants are distinct from the Chinese plants in the number of annular cells, regardless of ploidy level, but do not differ in spore ornamentation. Plants of Taiwan, Luzon and Seram differ from the Japanese and Chinese plants in the large muri on the perispore, and also from the Japanese plants in the number of annular cells. These variations suggest polyploidization after differentiation of the Japanese and Chinese populations, and a distinction between the temperate and tropical populations.

Key words: annular cells, chromosome number, cytogeography, *Gymnocarpium oyamense*, polyploidy, sporangium, spore.

Gymnocarpium Newman is a small genus of athyroid (= woodsoid) ferns distributed generally in cold temperate regions of the Northern Hemisphere. According to Sarvela (1978), the genus is divided into two subgenera, *Gymnocarpium* with five species and the monotypic *Currania*. Asian *Gymnocarpium oyamense* (Baker) Ching is the sole species of subgenus *Currania* and grows in rocky, often calcareous, areas in the mountains. Its main distribution range is within the Sino-Himalayan region from Nepal (alt. 2885 m) through China (300-2900 m) to Japan (500-1200 m), and also disjunctly southward at high elevations in Taiwan (e.g. Ali Shan, Kuan Shan), Luzon (2000-2900 m), the Philippines, Seram (2800-2900 m), Indonesia, and Papua New Guinea

(2400-3000 m) (Rosenstock 1913, Alston & Bonner 1956, Copeland 1960, Tsai & Shieh 1985, Kurata & Nakaike 1985, Kato 1994, Wang 1999) (Fig. 1).

Several species referable to *Currania* have been described from different localities; *Polypodium oyamense* Baker from Oyama, Kanagawa Prefecture, Japan, *Polypodium gymnogrammoides* Baker from Mt. Omei, Sichuan, China, *Currania gracilipes* Copel. from Luzon, the Philippines, and *Dryopteris genuflexa* Rosenst. from Mt. Bolan, Papua New Guinea (for taxonomic history see Wang 1999 and Copeland 1947). Ching (1933) concluded that all of these, except *Dryopteris genuflexa* not mentioned in his revision, are conspecific under *Gymnocarpium oyamense*. This taxonomy, also

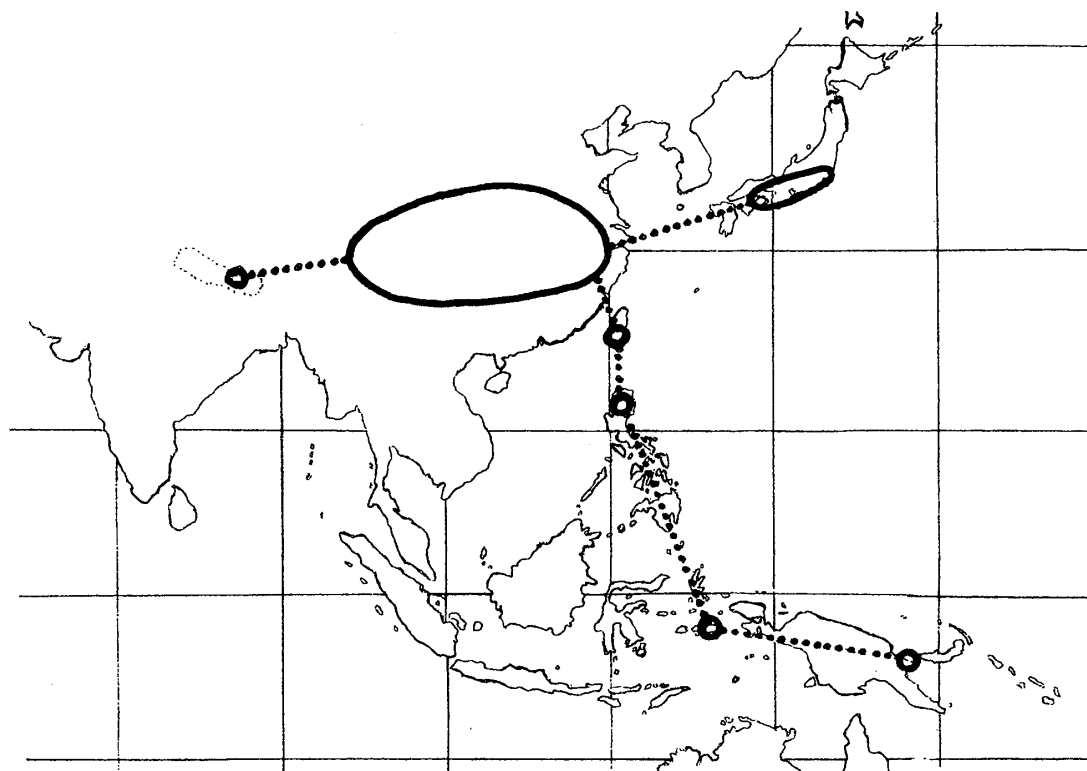


FIG. 1. Distribution range of *Gymnocarpium oyamense*.

including *Dryopteris genuflexa* as a synonym, has been followed in most recent taxonomic and floristic works (Ito 1939, Tagawa 1959, Nakaike 1975, 1992, Sarvela 1978, Kurata & Nakaike 1985, Salgado 1990, Shieh *et al.* 1994, Kato 1995, Wang 1999), although some workers recognized *Currania oyamensis* (Baker) Copel. within the independent genus *Currania* (Tagawa 1937, Alston & Bonner 1956, Ohwi 1957). Tagawa (1951) recognized two varieties in *Currania oyamensis*, var. *oyamensis* in Japan and China, characterized by short, straight sori (less than 3 mm long), and var. *gracilipes* in Luzon and Taiwan with longer, more or less falcate sori (to 4 mm long or a little longer) and with firmer leaf texture.

Chromosomal studies of *Gymnocarpium oyamense* from Japan and Taiwan revealed diploids with $n = 40$ chromosomes in Saitama, Japan (Mitui 1968, Hirabayashi 1969), and in Taichung, Taiwan (reported as var. *gracilipes*, Tsai & Shieh 1985), and

a tetraploid with $n = 80$ in Shizuoka, Japan (Kurita 1962). In the present study, we report three cytotypes; diploids, tetraploids and triploids, in Japan. We also report variation in the size and morphology of the spores, and in the number of annular cells in the sporangia on herbarium specimens, as well as cytological vouchers in relation to ploidy levels, and discuss the biogeography of *Gymnocarpium oyamense*.

Materials and Methods

Living materials were collected from five localities in the Kanto and Shikoku districts, Japan (Table 1). For somatic chromosome observations, root tips were pretreated in 2 mM 8-hydroxyquinoline for 5 hr, fixed in acetic alcohol (1:3) for 1 hr, and squashed in an aceto-orcein staining solution. The number of spores per sporangium was counted to infer reproductive mode. Cytological voucher spec-

TABLE 1. Chromosome number, spore length, and number of annular cells per sporangium in *Gymnocarpium oyamense*.

Locality	Chromosome no. (ploidy)	Spore length (mean, μm)	No. of annular cells (mean)
Previous studies			
Japan, Kanto, Saitama Pref., Chichibu, Futase (Mitui 1968)	$n = 40$ (2x)	—	—
Japan, Kanto, Saitama Pref., Chichibu, Ohchi-gawa (Hirabayashi 1969)	$n = 40$ (2x)	—	—
Japan, Chubu, Shizuoka Pref., Shizuoka-shi, Ohma (Kurita 1962)	$n = 80$ (4x)	—	—
Taiwan, Taichung, Kukuian (Tsai & Shieh 1985)	$n = 40$ (2x)	—	—
Origin unknown (Ogata 1928)	—	36 ^a	11 ^h
Philippines, Luzon, Benquet (Kremp & Kawasaki 1972)	—	44.0 ^{a, f}	—
China, Jiangxi (Chang <i>et al.</i> 1976)	—	43.5 ^{b, g}	—
Japan, cult. Bot. Gard., Univ. of Tokyo (Devi 1977)	—	36 ^{b, g}	—
Japan, Honshu; China, Hupeh; unknown origin (Sorsa 1980)	—	37.8 \pm 2.8 ^{b, g}	—
Japan, Nagano (Huang 1981)	—	32 - 37 ^{b, g}	—
Japan, Kanagawa Pref., Yamakita-machi, Yuhsein (Kurata & Nakaïke 1985)	—	—	9, 11 ⁱ
Japan, Tokyo Pref., Okutama-machi, Kori (Kurata & Nakaïke 1985)	—	ca. 32 ^{c, g}	—
Taiwan, Kaosiung, Kueiko-Yakou (Liu <i>et al.</i> 2000)	—	ca. 46 ^{d, f}	—
Present study			
Japan, Kanto, Saitama Pref., Hanno-shi, Mt. Izugatake, alt. 520 m (<i>Nakato</i> 2548)	$2n = 80$ (2x)	30.3 \pm 4.7 ^{e, g}	10.8 \pm 0.5
Japan, Kanto, Tokyo Pref., Okutama-machi, Nippara, alt. 660 m (<i>Nakato</i> 2552)	$2n = 80$ (2x)	27.6 \pm 2.7 ^{e, g}	10.8 \pm 0.7
Japan, Kanto, Kanagawa Pref., Kiyokawa-mura, Sakaizawa, alt. 720 m (<i>Nakato</i> 2567)	$2n = \text{ca. } 80$ (2x)	33.3 \pm 2.6 ^{e, g}	11.0 \pm 0.6
Japan, Shikoku, Kochi Pref., Monobe-son, Mt. Miune, alt. 900 m (<i>Nakato</i> 2511)	$2n = 160$ (4x)	35.7 \pm 3.5 ^{e, g}	10.9 \pm 0.7
Japan, Shikoku, Kochi Pref., Kahoku-cho, Matsuo-tohge, alt. 1050 m (<i>Nakato</i> 2554)	$2n = \text{ca. } 120$ (3x)	—	—

a: method for preparation not described. b: acetolysed, mounted in glycerine jelly. c: mounted in Canada balsam (one spore). d: SEM micrograph (one spore). e: mounted in Bioleit. f: length of spores, including perispores. g: length of spores, excluding perispores. h: one sporangium illustrated. i: two sporangia illustrated.

imens are deposited in TNS. Spores were directly mounted in Bioleit (Ohken Co., Tokyo), and the equatorial length along the long axis, excluding perispores, was measured using an ocular micrometer for 50 samples per cytological voucher specimen, and 20 samples for each herbarium specimen deposited in KYO, PE, PNH, PUH, TI, TNS and TOHO. For scanning electron microscopic (SEM) observations, spores were attached to specimen stubs covered with double-sided Scotch tape, spattered with gold, and observed using a Keyence, VE-7800, normally working at 20 kV. The number of annular cells in each sporangium was counted in

20 samples per specimen.

Results and Discussion

1. Chromosome number and reproductive mode

Chromosome counts of *Gymnocarpium oyamense* obtained in the present study, along with published data, are summarized in Table 1, Figs. 2 and 3. We observed that three plants from Tokyo, Saitama, and Kanagawa were diploid with $2n = 80$ or $2n = \text{ca. } 80$ ($x = 40$), one specimen from Kochi was tetraploid with $2n = 160$, and another from Kochi was triploid with $2n = \text{ca. } 120$, a new report in *Gymnocarpium*

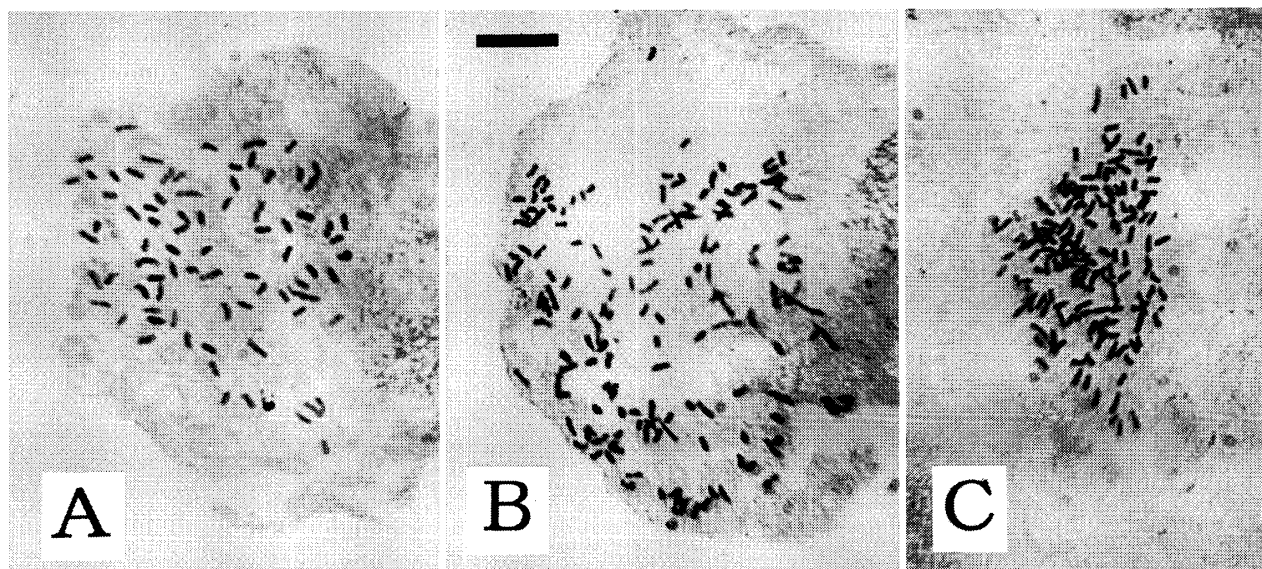


FIG. 2. Somatic chromosomes. A: Diploid, $2n = 80$ (*Nakato 2548*). B: Tetraploid, $2n = 160$ (*Nakato 2511*). C: Triploid, $2n = \text{ca. } 120$ (*Nakato 2554*). Scale bar : 10 μm .

oyamense (Fig. 2). The triploid may be a hybrid between a diploid and a tetraploid. The diploid and the tetraploid cytotype produced 64 brown bilateral spores per sporangium, while the triploid did not yield any spores due to incomplete or aberrant development of sporangia. Judging from the number of spores, the diploid and tetraploid plants are presumed to be sexual in reproductive manner, as reported by Kurita (1962), Mitui (1968), Hirabayashi (1969), and Tsai & Shieh (1985). The three cytotypes could hardly be distinguished from each other by frond morphology.

2. Spore and sporangium

2-1. Spore size

The mean length of the spores in the three diploid individuals ranged from 27.6 μm to 33.3 μm , while that in the tetraploid was 35.7 μm (Table 1, Fig. 4). The tetraploid therefore had larger spores than the diploids, although the difference was not obviously distinct. Spores size was examined on 105 herbarium specimens of *Gymnocarpium oyamense*, including 79 from Japan, 22 from China, 1 from Taiwan, 2 from Luzon (the Philippines), and 1 from Seram (Indonesia) (Table 2). In the Japanese specimens, the

variation in mean spore length was large, ranging from 27.0 μm to 41.0 μm (mean), certainly indicating a mixture of diploids and tetraploids. The link between spore size and ploidy level suggests that plants on most or all herbarium specimens with small spores (<34 μm , 24 specimens) are likely to be diploid, while specimens with larger spores (>35 μm , 46 specimens) are tetraploid. Spore lengths at the boundary between the two cytotypes may be around 34–35 μm (5 specimens). Additionally, four Japanese specimens had irregularly shaped spores, suggesting triploidy. The suspected triploid may be a rare F_1 hybrid.

In the Chinese specimens, the variation in mean spore length was as in the Japanese plants, ranging from 26.3 μm to 40.0 μm , certainly indicating that diploids and tetraploids also occur in China. Thirteen specimens had small spores (<34 μm , probably $2x$), seven had large spores (>35 μm , probably $4x$), and two had irregular spores (probably $3x$). A Taiwanese specimen had small spores (29.6 μm) and may be diploid. The two specimens from Luzon had spores 35.7 μm (probably $4x$) and 33.3 μm long (probably $2x$), respectively, and the Seram specimen had spores 35.5 μm long (probably

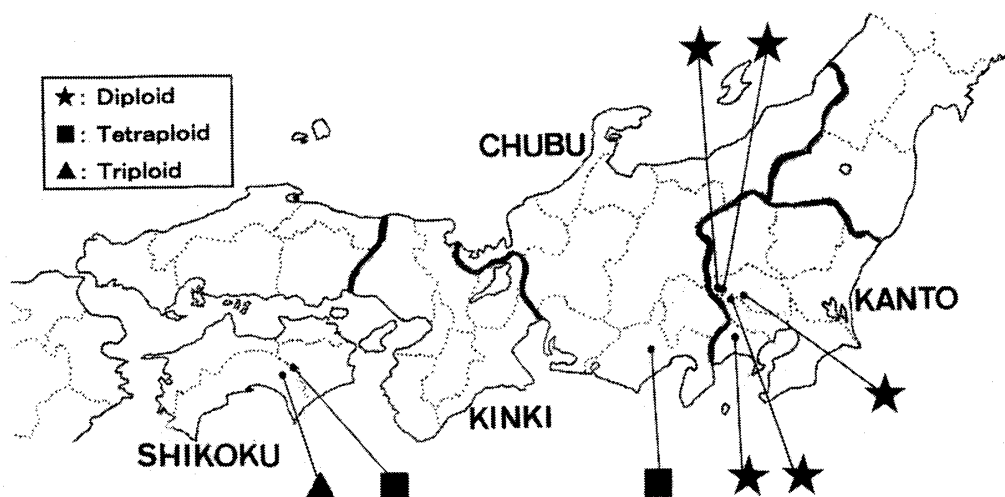


FIG. 3. Localities where cytological voucher specimens were collected.

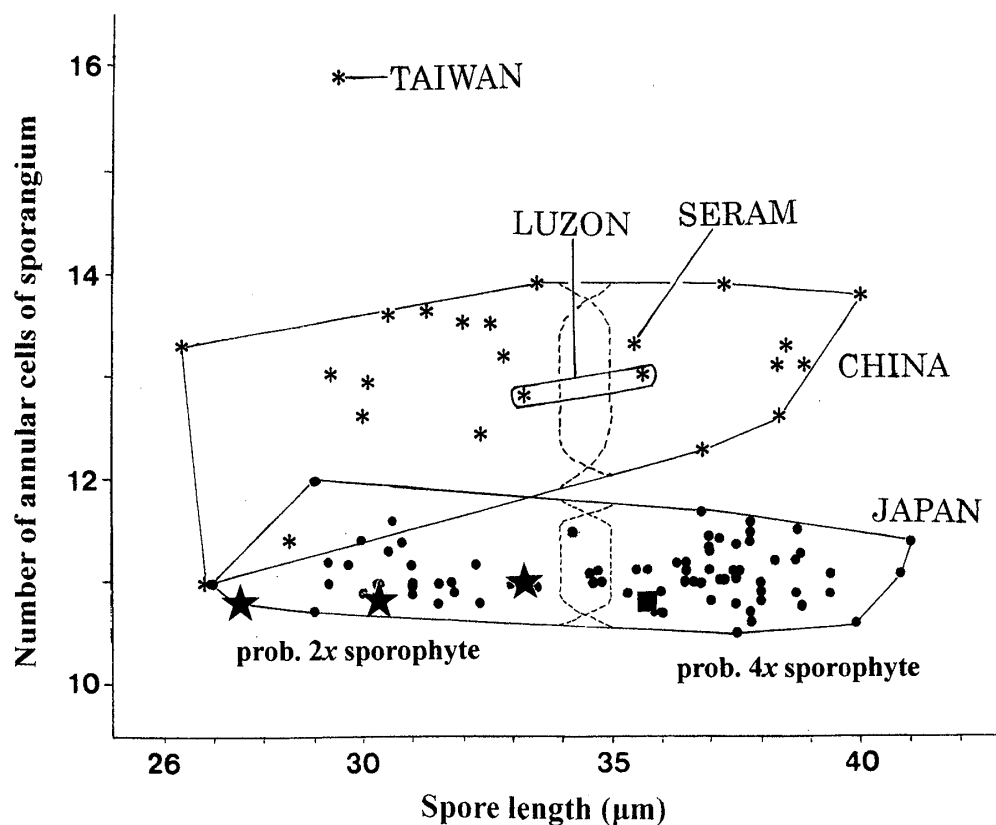


FIG. 4. Scatter diagram showing variation in mean spore length and mean number of annular cells of sporangium. Stars (★) and square (■) indicate diploid and tetraploid individuals, respectively. Dots (●) and asterisks (*) represent Japanese and extra-Japanese plants, respectively.

4x).

The spore size of *Gymnocarpium oyamense* has been reported by several workers (Ogata 1928, Kremp & Kawasaki 1972, Chang *et al.* 1976, Devi

1977, Sorsa 1980, Huang 1981, Kurata & Nakaike 1985, Liu *et al.* 2000). Those studies mostly used spores acetolysed and mounted in glycerin jelly, a treatment different from direct mounting in Bioleite

TABLE 2. Spore length and number of annular cells per sporangium as determined from herbarium specimens of *Gymnocarpium oya-mense*.

Locality	Specimen (herbarium)	Spore length (mean \pm SD; μ m)	Number of annular cells (mean \pm SD)
JAPAN; KANTO			
1. Ibaraki Pref; Kuji-gun, Daigo	<i>Shimizu & Yasu s.n.</i> (TNS 512014)	31.5 \pm 3.1	11.2 \pm 0.5
2. Ibaraki Pref., Kuji-gun, Daigo	<i>Nakazaki s.n.</i> (TNS 280875)	30.5 \pm 3.7	11.3 \pm 0.6
3. Gunma Pref., Tano-gun, Nakasato-mura	<i>Satomi s.n.</i> (TNS 569799)	irregular	—
4. Gunma Pref., Ueno-mura	<i>Mitsuhashi s.n.</i> (TNS 569798)	33.5 \pm 2.3	11.0 \pm 0.8
5. Saitama Pref., Mt. Mitsumine	<i>Tagawa 2234</i> (KYO)	irregular	—
6. Saitama Pref., Mt. Mitsumine	<i>Sahashi s.n.</i> (Sep. 1, 1973) (TOHO)	irregular	—
7. Saitama Pref., Mt. Buko	<i>Hisauchi 1342</i> (TI)	33.0 \pm 3.2	11.0 \pm 0.6
8. Saitama Pref., Ohtaki-mura, Mameyaki-zawa	<i>Ohba 635</i> (TI)	35.8 \pm 2.0	11.1 \pm 0.5
9. Saitama Pref., Ohtaki-mura, Takigawa	<i>Kobayashi s.n.</i> (TNS 527416)	37.5 \pm 2.8	11.1 \pm 0.7
10. Saitama Pref., Ohtaki-mura, Ohwa	<i>Waku 3063</i> (TNS)	36.0 \pm 2.8	10.9 \pm 0.7
11. Saitama Pref., Naguri-mura,	<i>Kobayashi s.n.</i> (TNS 527415)	30.0 \pm 2.8	10.9 \pm 0.9
12. Tokyo Pref., Nippara	<i>Hattori s.n.</i> (1937) (TI)	31.5 \pm 1.9	10.8 \pm 0.6
13. Tokyo Pref., Mt. Mitoh	<i>Takano s.n.</i> (TNS 170326)	29.3 \pm 2.2	11.0 \pm 0.5
14. Tokyo Pref., Hikawa-mura, Suniwa-zawa	<i>Mizushima s.n.</i> (Aug. 8, 1932) (TI)	29.3 \pm 2.2	11.2 \pm 0.7
15. Tokyo Pref., Ohme-shi, Kaminariki	<i>Satow 8783</i> (KYO)	29.7 \pm 2.6	11.2 \pm 0.6
16. Tokyo Pref., Okutama, Kori	<i>Sahashi s.n.</i> (Aug. 18, 1972) (TOHO)	30.6 \pm 2.0	11.6 \pm 0.6
17. Tokyo Pref., Okutama, Kori	<i>Satake s.n.</i> (TNS 324884)	31.0 \pm 2.0	10.9 \pm 0.6
18. Tokyo Pref., Okutama, Mt. Mitake	<i>Nakato 339</i> (TNS)	27.0 \pm 3.0	11.0 \pm 0.6
19. Tokyo Pref., Mt. Mitake	<i>Mizushima 57</i> (TI)	30.0 \pm 1.8	11.4 \pm 0.5
20. Tokyo Pref., Mt. Mitake - Hikawa	<i>Mizushima s.n.</i> (Oct. 20, 1946) (TI)	31.5 \pm 1.7	11.0 \pm 0.3
21. Tokyo Pref., Mt. Takao	<i>Sugawara s.n.</i> (TNS 521453)	29.0 \pm 2.4	12.0 \pm 0.6
22. Tokyo Pref., Takao, Kobotoke	<i>Azegami s.n.</i> (TNS 142392)	29.0 \pm 2.4	10.7 \pm 0.6
23. Kanagawa Pref., Atsugi, Miyagase	<i>Saito s.n.</i> (TNS 137254)	32.3 \pm 1.7	10.8 \pm 0.4
24. Kanagawa Pref., Oyama	<i>Hisauchi s.n.</i> (Aug. 22, 1915) (KYO)	31.8 \pm 3.1	11.0 \pm 0.3
25. Kanagawa Pref., Oyama	<i>Hisauchi s.n.</i> (Oct. 23, 1932) (TI)	31.0 \pm 1.9	11.0 \pm 0.6
26. Kanagawa Pref., Oyama	<i>Momiyama 437</i> (TI)	31.0 \pm 2.8	11.0 \pm 0.7
27. Kanagawa Pref., Oyama	<i>Nakato 2566</i> (TNS)	34.2 \pm 2.3	11.5 \pm 1.0
28. Kanagawa Pref., Oyama	<i>Moriya s.n.</i> (TNS 509102)	30.8 \pm 2.3	11.4 \pm 0.8
29. Kanagawa Pref., Afuri-jinja - Oyama	<i>Nakato 2568</i> (TNS)	31.8 \pm 3.1	10.9 \pm 0.4
30. Kanagawa Pref., Tanzawa, Yuhshin - Kurokura	<i>Ohba 10905</i> (TI)	38.0 \pm 2.3	10.9 \pm 0.6
31. Kanagawa Pref., Yamakita, Yuhshin	<i>Akiyama s.n.</i> (TNS 509543)	38.8 \pm 3.5	11.3 \pm 0.9
32. Kanagawa Pref., Nishi-tanzawa, Higashi-sawa	<i>Sugano s.n.</i> (TNS 146965)	30.3 \pm 2.2	11.0 \pm 0.5
JAPAN; CHUBU			
33. Yamanashi Pref., Mt. Kai-komagatake	<i>Okuyama 4072</i> (TNS)	36.5 \pm 2.5	11.1 \pm 0.6
34. Yamanashi Pref., Abe-tohge	<i>Okuyama s.n.</i> (TNS 89419)	37.0 \pm 2.9	11.3 \pm 0.7
35. Yamanashi Pref., Kamikuishiki-mura, Onna-zaka	<i>Kanai s.n.</i> (Sep. 15, 1957) (TI)	irregular	—
36. Yamanashi Pref., Kamikuissiki-mura, Aokigahara	<i>Hosokura s.n.</i> (TNS 586689)	37.5 \pm 1.9	10.8 \pm 0.6
37. Nagano Pref., Suwa-gun, Fujimi	<i>Yokouchi 326</i> (TNS)	36.8 \pm 2.3	11.0 \pm 0.8
38. Nagano Pref., Kitasaku-gun, Mochizuki	<i>Yamazaki s.n.</i> (TNS 572037)	37.0 \pm 1.9	10.8 \pm 0.6
39. Nagano Pref., Kizawa-mura, Nishisawado, alt. ca. 750m	<i>Iwatsuki 5958</i> (KYO)	38.7 \pm 3.1	11.5 \pm 0.6
40. Nagano Pref., Kizawa-mura, Kami-ishi	<i>Togashi s.n.</i> (Aug. 1, 1966) (TI)	34.8 \pm 2.3	11.0 \pm 0.8
41. Nagano Pref., Kizawa-mura, Osawado, alt. 900-1100 m	<i>Iwatsuki & Koyama 284</i> (KYO)	37.4 \pm 3.0	11.1 \pm 0.5
42. Nagano Pref., Kami-mura, Kitamatazawa	<i>Togashi s.n.</i> (Aug. 1, 1966) (KYO)	36.5 \pm 2.4	11.2 \pm 0.9

TABLE 2. continued.

Locality	Specimen (herbarium)	Spore length (mean \pm SD; μ m)	Number of annular cells (mean \pm SD)
43. Nagano Pref., Kami-mura, Kitamatazawa	<i>Togashi s.n. (Aug. 1, 1966) (TI)</i>	40.8 \pm 2.6	11.1 \pm 0.6
44. Nagano Pref., Ohshika-mura, Kamazawa, alt. 500-700 m	<i>Iwatsuki & Koyama 46 (KYO)</i>	34.5 \pm 3.2	11.1 \pm 0.7
45. Nagano Pref., Ohshika-mura, Koshibu-gawa	<i>Tsutsumi s.n. (TNS 550424)</i>	39.3 \pm 2.3	11.1 \pm 0.5
46. Nagano Pref., Ohshika-mura, Mt. Torikura	<i>Muramatsu s.n. (TNS 115554)</i>	37.5 \pm 1.5	10.5 \pm 0.6
47. Nagano Pref., Koshibu-gawa	<i>Futo 25500 (TNS)</i>	37.3 \pm 3.0	11.0 \pm 0.8
48. Nagano Pref., Minamimaki-miura	<i>Sato 643 (TI)</i>	35.5 \pm 2.5	11.1 \pm 0.4
49. Nagano Pref., Minamimaki-miura,	<i>Sato 1223 (TI)</i>	37.8 \pm 2.2	11.5 \pm 0.6
50. Shizuoka Pref., Gotenba-shi, Inno	<i>Hosokura s.n. (TNS 483520)</i>	37.0 \pm 3.1	11.4 \pm 0.6
51. Shizuoka Pref.; Ashitaka-yama-sankai, Tsurube-otoshi	<i>Kanai s.n. (Oct. 9, 1955) (TI)</i>	37.8 \pm 1.8	11.6 \pm 1.2
52. Shizuoka Pref., Suyama	<i>Sugimoto s.n. (TNS 246914)</i>	35.3 \pm 2.2	10.9 \pm 0.6
53. Shizuoka Pref., Abe-gun, Mafujiyama	<i>Kurosawa s.n. (Oct. 2, 1929) (KYO)</i>	36.6 \pm 2.3	11.0 \pm 0.6
54. Shizuoka Pref., Sumata -gawa	<i>Sugimoto s.n. (TNS 378596)</i>	38.3 \pm 2.0	11.2 \pm 0.8
55. Shizuoka Pref., Misakubo, Ohzore	<i>Yamashita s.n. (TNS 500683)</i>	36.0 \pm 2.9	10.7 \pm 0.6
56. Shizuoka Pref., Misakubo, Shirakura-gawa	<i>Sugino s.n. (TNS 500686)</i>	36.3 \pm 2.9	11.2 \pm 0.7
57. Gifu Pref., Mashita-gun, Osaka	<i>Suzuki s.n. (TNS 505612)</i>	36.8 \pm 2.2	11.7 \pm 0.8
JAPAN; KINKI			
58. Mie Pref., Oosugi-dani, Dokuranotaki	<i>Tagawa & Iwatsuki 1720 (KYO)</i>	35.9 \pm 3.3	10.7 \pm 0.7
59. Mie Pref., Oosugidani, Momonoki	<i>Tagawa 7299 (KYO)</i>	32.3 \pm 2.4	11.2 \pm 0.7
60. Mie Pref., Oosugidani, Momonoki	<i>Seto; 115218 (TNS)</i>	36.5 \pm 3.1	11.0 \pm 0.7
61. Mie Pref., Issi-gun, Exp. forests of Mie Univ.	<i>Seto s.n. (Jul. 15, 1950) (KYO)</i>	38.7 \pm 2.9	11.2 \pm 0.6
62. Nara Pref., Kashiwagi - Nishihara	<i>Koidzumi s.n. (Jul. 14, 1922) (KYO)</i>	36.9 \pm 2.4	11.3 \pm 0.6
63. Nara Pref., Yoshino, Tengawa-mura	<i>Tagawa 5785 (KYO)</i>	39.9 \pm 2.0	10.6 \pm 0.6
64. Hyogo Pref., Mt. Rokko, Arimagawa	<i>Mizutani s.n. (Jul. 2, 1953) (KYO)</i>	34.7 \pm 3.0	11.1 \pm 0.5
65. Hyogo Pref., Mt. Rokko, Ura-Rokko, alt. ca. 500 m	<i>Iwatsuki 1320 (KYO)</i>	37.1 \pm 2.8	11.4 \pm 0.5
JAPAN; SHIKOKU			
66. Tokushima Pref., Mt. Tsurugi	<i>Yamashita s.n. (Jul. 19, 1929) (KYO)</i>	38.4 \pm 2.9	10.9 \pm 0.3
67. Tokushima Pref., Mt. Tsurugi	<i>Nikai; s.n. (Aug. 26, 1908) (TI)</i>	38.0 \pm 2.9	11.0 \pm 0.6
68. Tokushima Pref., Kitoh-son	<i>Abe s.n. (TNS 476180)</i>	38.8 \pm 3.5	10.8 \pm 0.5
69. Tokushima Pref., Higashi-iyayama-son	<i>Abe s.n. (TNS 475921)</i>	37.8 \pm 1.8	10.6 \pm 1.1
70. Ehime Pref., Doi-machi, Iratsu	<i>Ishikawa 67 (KYO)</i>	41.0 \pm 3.1	11.4 \pm 0.7
71. Ehime Pref., Omogo-mura, alt. 700 m	<i>Murata 9313 (KYO)</i>	34.5 \pm 3.3	11.0 \pm 0.9
72. Ehime Pref., Omogo	<i>Yamashita s.n. (Aug. 2, 1930) (TI)</i>	37.8 \pm 2.8	10.7 \pm 0.6
73. Ehime Pref., Mt. Ishizuchi	<i>Ogata s.n. (Aug. 7, 1925) (KYO)</i>	38.7 \pm 2.2	10.9 \pm 0.6
74. Ehime Pref., Kamo-mura, Kawagurusu	<i>Yamamoto s.n. (Aug. 20, 1934) (TI)</i>	37.5 \pm 2.7	11.4 \pm 0.8
75. Kochi Pref., Kajiga-mori	<i>Morita s.n. (Aug. 18, 1939) (TI)</i>	37.5 \pm 3.5	11.1 \pm 0.7
76. Kochi Pref., Awa-gun, Mt. Tsutsujo-zan	<i>Kato 178 (KYO)</i>	36.9 \pm 2.8	11.1 \pm 0.4
77. Kochi Pref., Agawa-gun, Tsubayama-mura	<i>Makino s.n. (1885) (TI)</i>	37.3 \pm 2.8	11.0 \pm 0.8
78. Kochi Pref., Awa-gun, Nanokawa	<i>anonymous (Jul. 11, 1892) (TI)</i>	37.8 \pm 2.6	11.4 \pm 0.6
79. Kochi Pref., Agawa-gun, Yasui-mura	<i>Yoshinaga s.n. (Aug., 1887) (TI)</i>	38.0 \pm 2.9	11.0 \pm 0.6
CHINA			
80. Anhui, Vicinity of Sze Tze Koo	<i>P.-C. Toong 4039 (PE)</i>	36.8 \pm 2.8	12.3 \pm 1.2
81. Jiangxi, Tonggu	<i>Y.-G. Xiong s.n. (PE 400485)</i>	38.5 \pm 1.7	13.3 \pm 1.7
82. Jiangxi, Mt. Mubu	<i>Y.-G. Xiong 5948 (PE)</i>	38.8 \pm 2.8	13.1 \pm 1.4
83. Jiangxi	<i>anonym. (PE 400486)</i>	38.3 \pm 3.3	12.6 \pm 1.3
84. Henan, Songxian	<i>Henan Forestry Dept. 1078 (PE)</i>	32.8 \pm 2.8	13.2 \pm 1.0
85. Hunan, Xinningxian, Mt. Ziyunshan	<i>L.-H. Liu 15344 (PE)</i>	38.3 \pm 2.4	13.1 \pm 1.3
86. Sichuan, Tianquan	<i>K.-S. Shing 5482 (PE)</i>	26.3 \pm 3.1	13.3 \pm 1.3
87. Sichuan, Mt. Omei, alt. 2450 m	<i>K.-S. Shing & K.-Y. Lang 274 (PE)</i>	37.3 \pm 1.7	13.9 \pm 1.2
88. Sichuan, Mt. Omei, alt. 1810 m	<i>K.-S. Shing & K.-Y. Lang 719 (PE)</i>	33.5 \pm 2.1	13.9 \pm 2.0
89. Sichuan, Mt. Omei, alt. 2400 m	<i>K.-S. Shing & K.-Y. Lang 215 (PE)</i>	32.3 \pm 2.1	12.4 \pm 1.1
90. Sichuan, Mt. Omei, alt. 2200 m	<i>Z.-R. Wang & G.-M. Xu 18 (PE)</i>	32.5 \pm 3.2	13.5 \pm 1.2

TABLE 2. continued.

Locality	Specimen (herbarium)	Spore length (mean \pm SD; μ m)	Number of annular cells (mean \pm SD)
91. Sichuan, Mt. Omei, alt. 2800 m	<i>T.-C. Peng 603</i> (PE)	32.0 \pm 2.9	12.5 \pm 0.9
92. Sichuan, Mt. Omei	<i>H.-C. Chow 7941</i> (PE)	30.0 \pm 3.1	12.6 \pm 1.4
93. Sichuan, Baoxing, alt. 1700 m	<i>X.-S. Zhang & Y.-C. Ren 755</i> (PE)	irregular	—
94. Guizhou, Nayong, Longchang	<i>F. Wang 90219</i> (PE)	irregular	—
95. Guizhou	<i>P.-H. Wang s.n.</i> (PE 571502)	40.0 \pm 3.5	13.8 \pm 0.5*
96. Yunnan, Luquan	<i>W.-M. Chu 1567</i> (PE)	26.8 \pm 1.2	11.0 \pm 1.0
97. Yunnan, Lijang	<i>K.-M. Feng 2626</i> (PE)	28.5 \pm 2.0	11.4 \pm 0.9
98. Yunnan, Lijang	<i>K.-M. Feng 9508</i> (PE)	29.3 \pm 2.0	13.0 \pm 1.2
99. Shaanxi, Foping	<i>P.-C. Tsoong 3320</i> (PE)	30.5 \pm 2.3	13.6 \pm 1.6
100. Gansu, Kangxian	<i>Y.-S. Lian 96513</i> (PE)	30.1 \pm 2.9	12.9 \pm 1.2
101. Gansu, Kangxian	<i>Y.-P. Xu 1038</i> (PE)	31.3 \pm 1.9	13.6 \pm 1.2**
TAIWAN			
102. Mt. Arisan, Kodamayama to Sekisan	<i>Tatewaki s.n.</i> (Mar. 24, 1932) (KYO)	29.6 \pm 2.6	15.9 \pm 2.1
PHILIPPINES; LUZON			
103. Luzon, Benquet, Kabayan municipality, alt. 2430 m	<i>L. Co 3907</i> (PUH)	35.7 \pm 2.8	13.0 \pm 0.7
104. Luzon, Mt. Plog, alt. 2900 m	<i>anonymous</i> (PNH 63163)	33.3 \pm 3.6	12.8 \pm 0.9
INDONESIA; SERAM			
105. Seram, Manusela Natl. Park, Mt. Binaiya, Owac Puku	<i>Kato et al. C-3851</i> (TI)	35.5 \pm 2.4	13.3 \pm 0.9

* calculated from four samples. ** calculated from nine samples.

used in the present study. Because spores mounted in glycerin jelly tend to swell (Pryer & Britton 1983), we could not simply compare the reported spore sizes and those measured in this study. In contrast, one spore mounted in Canada balsam (Kurata & Nakaike 1985; Japan, Okutama-machi) was ca. 32 μ m long, excluding the perispore, and presumed to be from a diploid plant. One SEM figure of a spore from Taiwan, Kaosiung (Liu *et al.* 2000), was ca. 46 μ m long, including the perispore, which corresponds to the tetraploid cytotype. Therefore, two cytotypes, diploid and tetraploid, may occur in Taiwan.

2-2. Spore morphology

SEM observations of spores from voucher specimens revealed that the perispore surface of the diploids is crumpled and with short perforated folds or muri (Fig. 5A), while that of the tetraploid is tuberculate and sometimes with very small pits at the base of the projections (Fig. 5B). Spores of some herbarium specimens were observed by SEM

and compared with those of the cytological vouchers. Suspected diploid and tetraploid specimens from Japan (nos. 31, 32, 45 & 68 in Table 2) and China (nos. 84, 85, 88 & 96) showed basically similar perispore morphologies, sometimes with slight variation (Fig. 5D). The perispores of the extra-Japanese (Taiwan, Luzon, and Seram) specimens had larger and longer perforated folds and more developed large muri than the Japanese diploids and tetraploids (Fig. 5C, E, F).

We noted that two micrographs of the spores of *Gymnocarpium oyamense* reported by Tryon & Lugardon (1991; p. 490, figs. 12 and 13), one from Sichuan, China, and the other from Japan, closely resemble those of the diploids and tetraploid observed in our study, respectively, in both size and perispore morphology. The Chinese and Japanese materials are therefore probably diploid and tetraploid, respectively. Liu *et al.* (2000) reported verrucate ornamentation with a few caves, as we found in diploids, in a Taiwanese individual of *Gymnocarpium oyamense*, but in size the spores

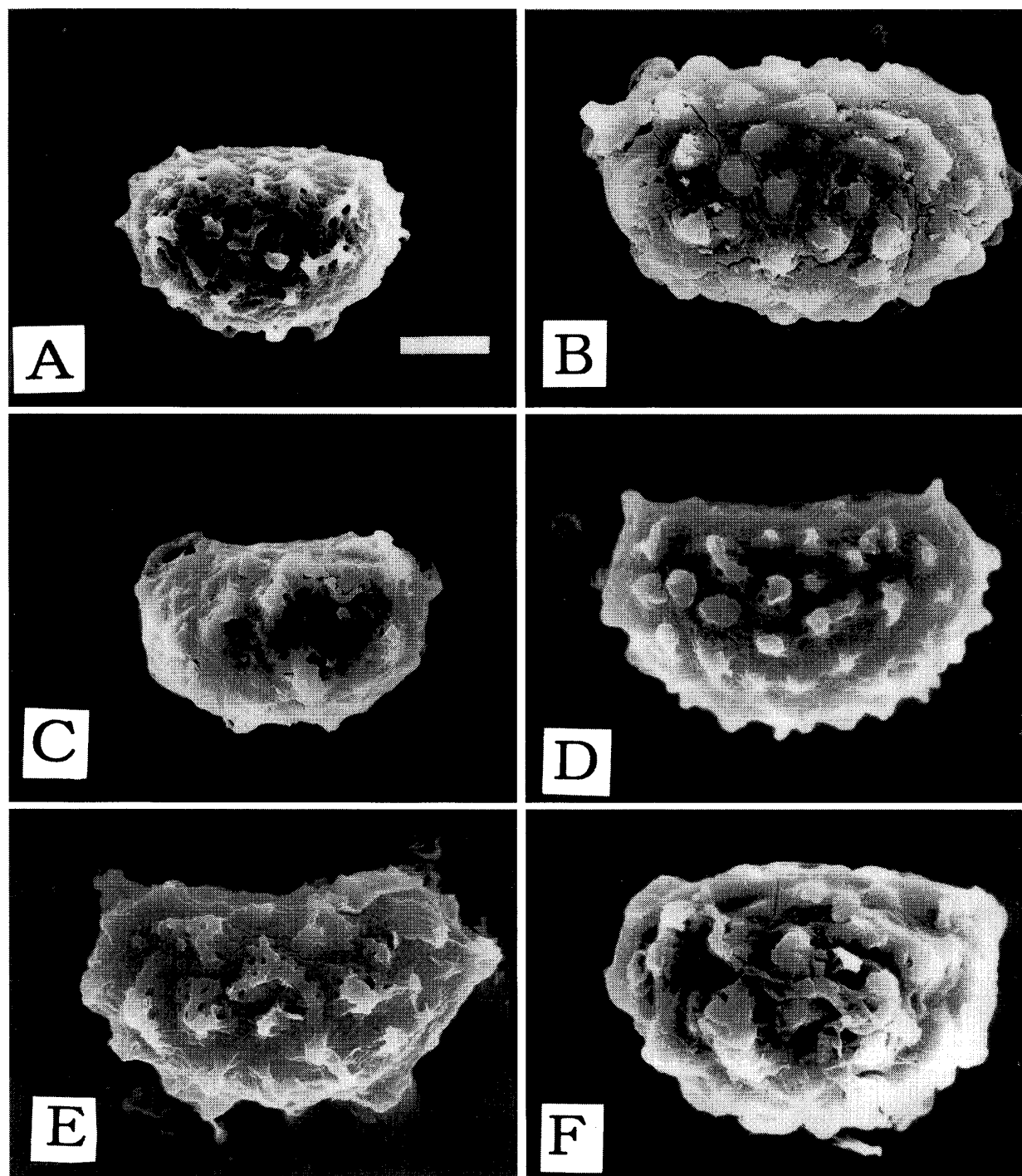


FIG. 5. SEM micrographs of spores. A, B: Cytological voucher specimens from Japan. A: Diploid, *Nakato* 2552. B: Tetraploid, *Nakato* 2511. C-F: Herbarium specimens. C: No. 102, Taiwan, *Tatewaki* s.n. (KYO), probably 2x. D: No. 68, Japan, *Abe* s.n. (TNS 476180), probably 4x. E: No. 103, Luzon, *L. Co* 3907 (PUH), probably 4x. F: No. 105, Seram, *Kato et al.* C-3851 (TI), probably 4x. Scale bar : 10 μ m.

correspond to the tetraploid as described above. Sorsa (1980) reported smooth or slightly patterned perispore surfaces in spores of *Gymnocarpium oya-*

mense. We did not, however, observe such ornamentation in the materials we examined.

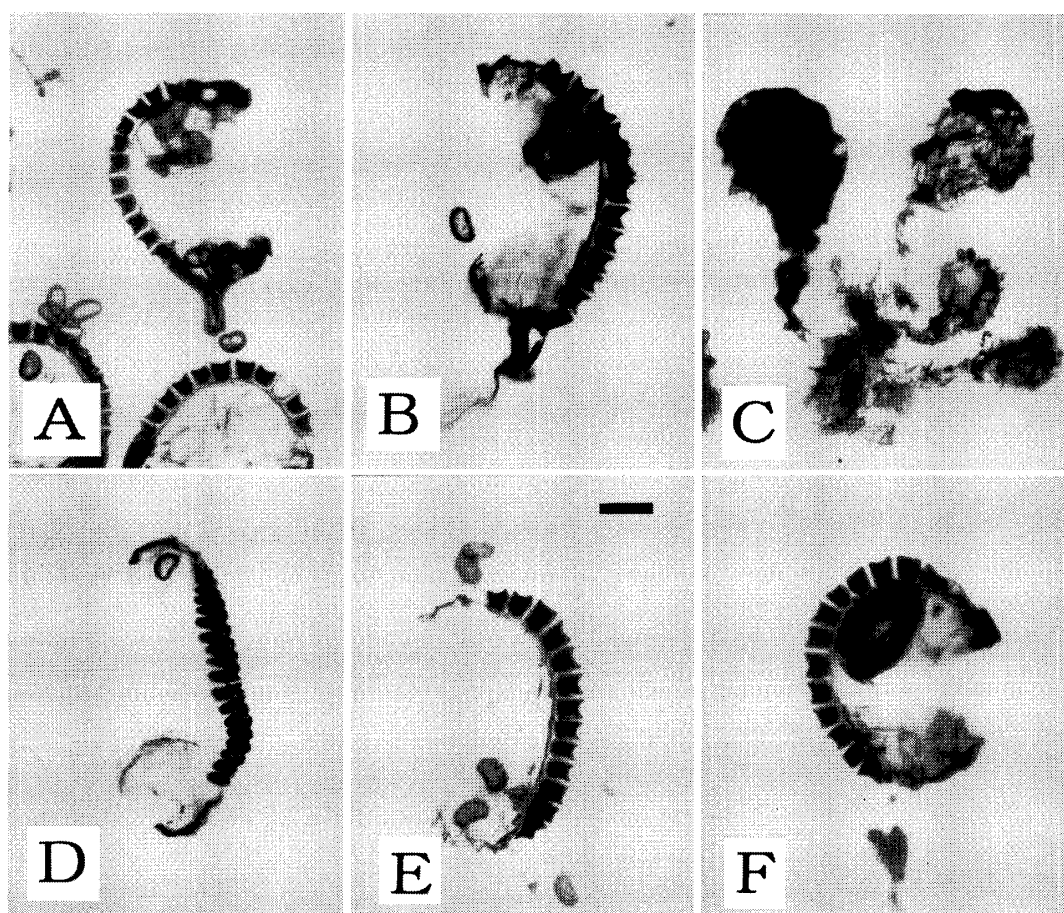


FIG. 6. Dehiscent sporangia (except those in C). A-C: Cytological voucher specimens from Japan. A: Diploid with 11 annular cells, *Nakato 2552*. B: Tetraploid with 11 annular cells, *Nakato 2511*. C: Triploid with aberrant sporangia, *Nakato 2554*. D-F: Herbarium specimens. D: No. 102, sporangium with 18 annular cells; Taiwan, *Tatewaki s.n.* (KYO). E: No. 103, sporangium with 13 annular cells; Luzon, *L. Co 3907* (PUH). F: No. 105, sporangium with 13 annular cells; Seram, *Kato et al. C-3851* (TI). Scale bar: 50 μ m.

2-3. Annular cells of sporangium

The number of annular cells in the sporangia was counted in the diploid and tetraploid vouchers. The mean number of cells in the diploid individuals was 10.8-11.0; the mean number in the tetraploid was 10.9, indicating that no difference exists between the two cytotypes (Table 1, Figs. 4 & 6).

The number of annular cells in the sporangia was examined in the same herbarium specimens as used to measure spore size (Table 2, Fig. 4). The annular cells per sporangium in the Japanese specimens were fairly constant in number, 10.6-12.0 (mean), regardless of differences in spore size. By contrast, specimens from China (11.0-13.9, usu-

ally more than 12.3), Luzon (12.8, 13.0) and Seram (13.3) have more annular cells than do the Japanese plants. The Taiwanese specimens have many annular cells (15.9). Copeland (1947) also reported about 14 cells per annulus in plants from Luzon.

3. Cytogeography

The range of distribution of the diploid and tetraploid cytotypes of *Gymnocarpium oyamense* was inferred from spore size of herbarium specimens, which were categorized into three size classes, as described above. In Japan, most specimens from the Kanto district, i.e. Ibaraki, Gunma, Saitama, Tokyo and Kanagawa Prefectures, have spores short-

er than 34 μm , while most specimens from western Japan, i.e. the Chubu, Kinki, and Shikoku districts, have spores longer than 35 μm (Fig. 7A). The data suggest that the presumed diploid and tetraploid cytotypes are generally allopatric in Japan; the former occurs in Kanto, while the latter occurs west of Kanto. The type specimen of *Gymnocarpium oyamense* is thought to be diploid, because all five herbarium specimens collected at the type locality have small spores, 30.8–34.2 μm long (mean) (nos. 24–28 in Table 2). Presumed tetraploids with large spores may also occur in some sites in western Kanto, i.e. Ohtaki-mura in Saitama Prefecture (nos. 8, 9 & 10) and Yushin in Kanagawa Prefecture (nos. 30 & 31). It was observed that suspected triploids producing abortive spores were found in Nakasato-mura (no. 3) and on Mt. Mitumine (nos. 5 & 6), both close to Ohtaki-mura. Diploids and tetraploids, therefore, are likely to coexist in these areas and hybridization occurred between them. Because of the existence of a specimen with small spores (no. 59) from Kinki (Mie Prefecture), and a triploid, *Nakato* 2554, from Shikoku (Kochi Prefecture), it is also possible that the diploid cytotype, even though rare, may occur in Kinki and Shikoku, where tetraploids are common. In mainland China, suspected diploids have a relatively wide distribution range in inland provinces, i.e. Henan, Sichuan, Yunnan, Shaanxi and Gansu, while the suspected tetraploids occur in a narrower range in the eastern provinces of Anhui, Jiangxi, Guizhou and Hunan, and also in Sichuan (Fig. 7B).

Thus, presumed diploids are distributed disjunctly in the Kanto area, Taiwan, and in the inland provinces of China, while presumed tetraploids occur in western Japan and eastern-central China. The Japanese and Chinese populations differ in the number of annular cells, suggesting differentiation in the two. It may be likely, therefore, that the tetraploids arose independently in Japan and China. After tetraploids derived from diploids somewhere in the western part of Japan, the tetraploids may

have expanded and replaced the diploids, but barely invaded the Kanto and Tanzawa Mountains, except along the western margin of the Mountains.

Geologically, the Kanto area where the diploids and suspected diploids occur is characterized by Mesozoic to Tertiary formations; it includes the Kanto and Tanzawa Mountains of Gunma, Saitama, Tokyo, Kanagawa Prefectures, and the Yamizo Mountains, Ibaraki Prefecture. In the Pleistocene period, the northern, western and southwestern circumferences of the Kanto to Tanzawa Mountains became gradually surrounded by recently erupted volcanoes, such as Mt. Haruna (ca. 300,000 yrs ago - present), Mt. Asama (ca. 100,000 yrs ago -), Yatsugatake (ca. 1 million yrs ago -), Mt. Fuji (ca. 80,000 yrs ago -), and Mt. Hakone (ca. 500,000 yrs ago -) (Kaneoka *et al.* 1980, Yamashita 1995, Kaizuka *et al.* 2000). Those volcanic eruptions may have isolated diploid populations in the Kanto, Tanzawa and Yamizo Mountains from the tetraploid populations in the western localities.

Gymnocarpium oyamense is primarily a Sino-Japanese element widely distributed in China and Japan, but extending southward to Taiwan, Luzon, Seram and Papua New Guinea, areas that are distant from each other (Fig. 1). Southward expansion may have occurred through the Formosa-Luzon migratory track (van Steenis 1934) or a pathway linking Taiwan and New Guinea through the Philippines and North Moluccas, which was uplifted within the last 5 million years (Hall 1998). Isolation among populations on the southern islands may be a relict of past wider distribution or, more likely, the result of long distance dispersal. Such an event took place at high elevations on the tropical islands, e.g. Seram Island, which emerged as an island 3–6 million years ago (Audley-Charles 1993): high elevations became available later.

Plants in the southern, tropical regions differ from those of Japan and China in perispore ornamentation and sorus length (Tagawa 1951), suggesting a more distinct isolation from the north-

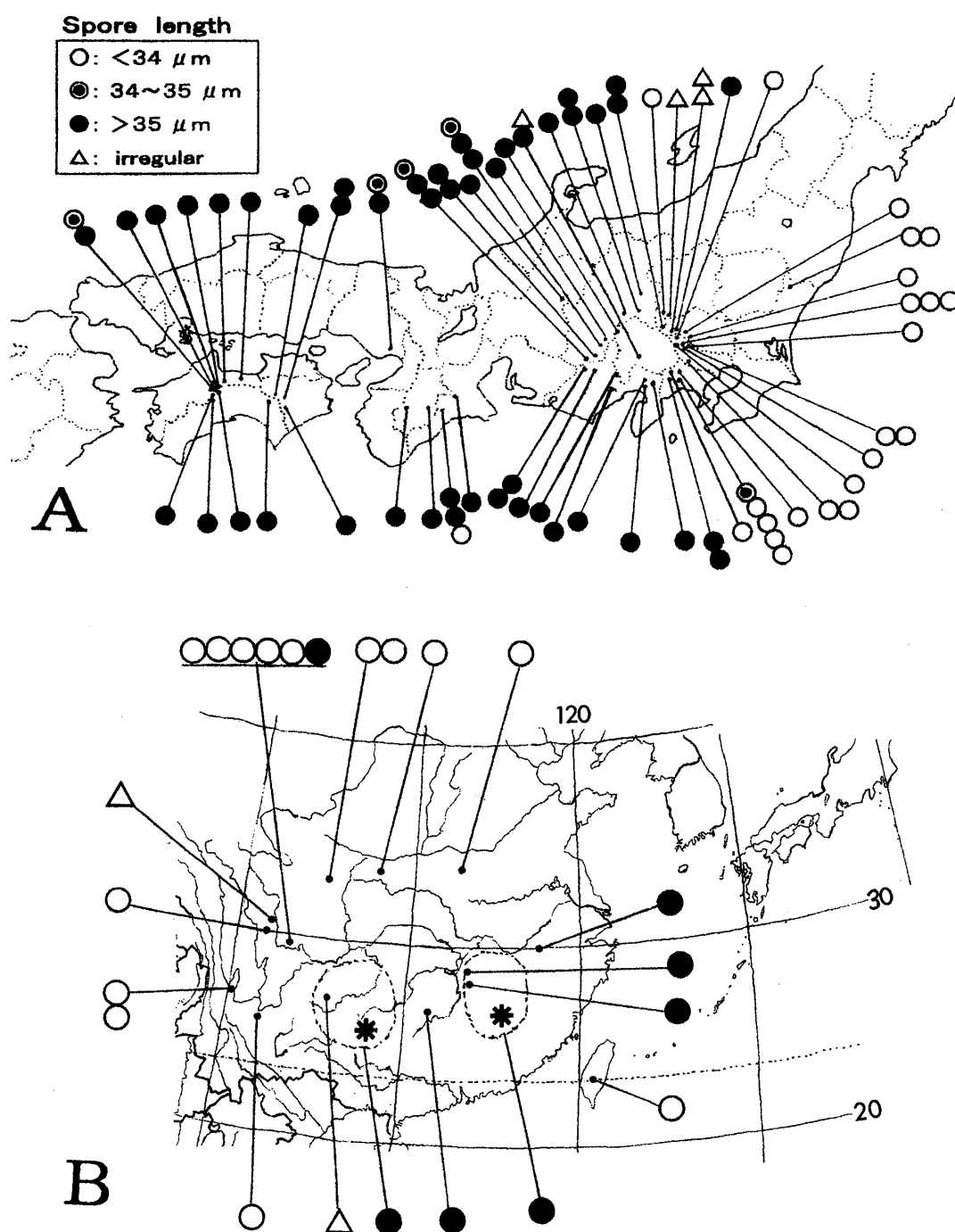


FIG. 7. Localities where herbarium specimens were collected, based on mean spore length in Japan (A), and China and Taiwan (B). The places of collections for nos. 85 (Jiangxi) and 95 (Guizhou) are uncertain and are indicated by asterisks (*). Areas enclosed by dotted lines roughly correspond to Jiangxi (right) and Guizhou (left) provinces.

ern, temperate populations than the disjunction between the Japanese and Chinese populations. In particular, the morphologically (also in the number of annular cells) most distinctive Taiwanese diploids

may have been strongly isolated, but their relationship to diploids and tetraploids in the neighboring regions remains uncertain. Further comparative cytological, morphological and molecular studies are

needed to provide a better understanding of the taxonomy and historical biogeography of the *Gymnocarpium oyamense* complex.

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